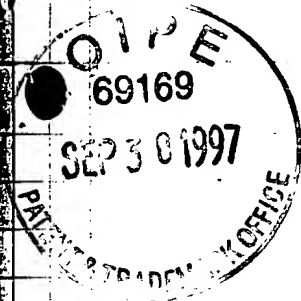


RECEIVED

OCT 15 1997



CS 11.1 Good expansion of "flat" ES-like colonies; however endoderm differentiation taking place more abundantly. Only clone at good feeders (ie no over-growth)

CS 11.2 Over-grown by feeders that were not properly inactivated. Very few ES-like colonies recognizable

CS 11.3 Also cont. w/ non-inactivated STO cells. Some very good ES-like colonies remain though.

CS 11.4 Many endoderm; a few ES-like colonies.

- ① Added Ca²⁺ / Mg²⁺ free pos to each of above; chose individual colonies, tag w/ 5" → chose individual cells
 → 96 well plates w/ 3T3 cells.
 did 248) Line [half has bFGF & h Steel factor. (Young)]
 → 5% CO₂ / 5% O₂
 N.B. manipulations took a long time & pH in medium changed. (expect poor cloning efficiency if any)

- ② Split CS 11.1 → T25 w/ 3T3 cells.

→ Will try to pass off fibroblasts/endoderm by allowing split ES cells to attach & keep floating after 15-20 minutes

4x T75 = 2 T75

2x T25 = 1.5 T25

[10 wells]
 [5+4]

① bFGF + h Steel factor 96 well plates: No colonies

② ES 96 well plate ② colonies CS 11.1, 3, 4

① colony CS 11.2

Added new 3T3 cells w/ moving colonies.

- ③ Split CS 11.1 → 2 T75s w/ 3T3 cells on 6/6/93

Some endoderm differentiation. (bad in one flask)

- ④ Individual colonies w/ feeders of CS 11.3 & CS 11.4 still undifferentiated.

- ⑤ "Panning" did not work the 6 well plate of CS 11.3 was very over-grown. However, many good colonies remained. AP/Rxn (Donovan, 1986) ④

BEST AVAILABLE COPY